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Subject. Materials for interview; Application 09/304,121

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Application 09/304,121

For discussion at the interview

Proposed amended claim 1, solution A

1. A molecular circuit comprising (a) a first nucleic acid molecule that comprises a gene encoding a transcription factor, **with the proviso that the transcription factor is not identical with an endogenous heat shock transcription factor**, and a first promoter activatable by stress and by the transcription factor, wherein the first promoter and the transcription factor gene are operably linked, and (b) a second nucleic acid molecule that comprises a gene of interest and a second promoter activatable by the transcription factor, wherein the second promoter and the gene of interest are operably linked.

In the Office Action dated June 27, 2001, the Examiner maintained her rejection of claim 1 under 35 U.S.C. 102(b) as being anticipated by Moonen Chrit (WO 98/06864). The Examiner stated that "Moonen Chrit discloses activating endogenous heat shock genes in a mouse, which has been transformed with an adenoviral vector. The reference discloses a therapeutic gene under the control of a heat shock promoter. The therapeutic gene and heat shock promoter combination is interpreted as the second nucleic acid molecule while the first nucleic acid molecule is interpreted to be the endogenously present heat shock promoter and heat shock transcription factor. Therefore, the instant invention is anticipated by Moonen Chrit." The Examiner suggested that claim 1 could be rescued by an amendment that indicates "that the nucleic acid of the molecular circuit must be inserted into the cell".

Accepting Examiner's argument for the sake of speedily completing prosecution, Applicant would like to observe that the Examiner's proposed remedy casts too wide a net. In addition, amendment of claim 1 as suggested would necessitate introduction of at least one additional independent claim and changes in many dependent claims. Applicant would like to point out that the concern of the Examiner is specific to the situation in which element (a) includes an endogenous heat shock transcription factor. Only in this case would element (b) comprise a gene of interest linked to a heat shock promoter, i.e., a construct of the type anticipated by Moonen Chrit. If element (a) included another

transcription factor, e.g., NFkB, a molecular circuit of the invention could only be constituted by providing an element (b) comprising a gene of interest controlled by a promoter activatable by NFkB, i.e., a construct different from Moonen Chrit's heat shock promoter-containing constructs. Applicant therefore argues that the proper remedy that will place claim 1 into condition for allowance is an amendment of the claim that excludes the possibility that the transcription factor of element (a) can be identical with an endogenous heat shock transcription factor. Claim 1 has now been amended by the inclusion of a proper proviso.

Support for the proviso can be found in the specification. The term "endogenous HSF" appears in several places in the specification, i.e., on p.12, line 13, p.19, line 25, and p.20, lines 3-4 and 13. While for reasons that should become obvious below the specification lacks a direct statement that the transcription factors of the invention do not include endogenous heat shock transcription factor, this is implied based both on logic and formalistic grounds.

1 Logic grounds

As is well known in the field and is implied in the description provided in the paragraph bridging pp.1 and 2 of the specification, heat shock promoters are not directly activated by a physical or chemical stress. They are activated by the intermediary of an activated heat shock transcription factor binding to HSE sequences present in the promoters. This is a fact not a hypothesis: deletion of HSE sequences renders a heat shock promoter incapable of being stress activated, and deletion of the heat shock transcription factor 1 gene in a knockout mouse renders heat shock promoters unresponsive to stress activation. Hence, activation by stress of a heat shock promoter requires a preexisting heat shock transcription factor that is activatable by the stress. If the expression of endogenous heat shock transcription factor were controlled by a heat shock promoter, no heat shock promoter could be activated by stress (because heat shock transcription factor would not be present at the time of stress).

The molecular circuits of the present invention have general characteristics recited in several places in the specification. The title of the application reads "Molecular regulatory circuits to achieve sustained activation of genes of interest by a single stress". The first paragraph on p.12 reads "Several regulatory circuits are described herein that have the general feature that their use permits sustained activation of expression of a gene of interest by a single application of stress. Gene activity regulated by these circuits does not

subside subsequent to the activating stress." The circuit the Examiner fears may be improperly included in claim 1 consists of

(a) a first nucleic acid molecule that comprises the endogenous gene encoding heat shock transcription factor 1 and a first promoter activatable by stress (a heat shock promoter), wherein the first promoter and the transcription factor gene are operably linked, and (b) a second nucleic acid molecule that comprises a gene of interest and a second promoter activatable by the transcription factor (a heat shock promoter), wherein the second promoter and the gene of interest are operably linked.

As discussed above, if the endogenous HSF1 gene were controlled by a heat shock promoter, prior to activation of the latter heat shock promoter, the cell will not contain HSF1, and HSF1 is required for activation of the heat shock promoter. Hence, the regulatory circuit will not be activatable by a stress and, therefore, is not a circuit of the invention. (In fact, it is not a regulatory circuit at all, but is inert nucleic acid material.)

2 Formalistic grounds

In the specification, the molecular circuits of the invention are characterized as being of three different types, types 1, 2 and 3.

The transcription factor component of a type 1 circuit is a mutated heat shock transcription factor, which is different from endogenous heat shock transcription factor (p.12, line 8).

Furthermore, the separate function of endogenous heat shock transcription factor is described specifically for type 1 and 2 circuits, and in shorthand form for type 3 circuits. See p.12, lines 12-14: "When the cells are stressed, promoters in both elements are activated by endogenous HSF, which results in the expression and accumulation of the gene product of interest and of mutated HSF."

The transcription factor component of a type 2 circuit is a mutated heat shock transcription factor in which the HSE DNA-binding domain has been replaced by that of another factor. See p.19, lines 14-17: "The illustrative type 2 circuit shown in Figure 2 differs from a type 1 circuit in three ways. First, the type 2 circuit contains a mutated HSF in which the HSE DNA-binding domain has been replaced by that of bacterial repressor

LexA. This substituted factor no longer binds stress promoters but promoters containing LexA recognition sites."

For the separate role of endogenous HSF, see p.20, lines 3-4: "A type 2 circuit operates as follows. After transient stress, endogenous HSF is activated, which results in expression and accumulation of mutated HSF." See also p.21, lines 5-6: "The transcription factor gene is controlled by a promoter that can be activated by the mutated HSF and by endogenous HSF."

For the transcription factor component of a type 3 circuit, see p.21, lines 16-18: "Other forms of circuits can be constructed in which the transcription factor is not mutated HSF. That is, any constitutively active transcription factor can be used in lieu of mutated HSF." Note that endogenous HSF is continually expressed, but is not constitutively active. It is only active transiently during a stressful event. Hence, endogenous HSF cannot be a transcription factor component of a type 3 circuit.

For a separate role of endogenous HSF, see the description of an example type 3 circuit on p.22, lines 17-20: "This form of type 3 circuit operates as follows. In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate one set of transcription factor genes,..." Thus, a transient stress will activate the heat shock promoter controlling the transcription factor genes. The transcription factor genes cannot be endogenous HSF, because activation of the genes requires preexisting endogenous HSF.

Finally, note that the stated purpose of the invention is to proceed beyond the simple uses of heat shock promoters that were discussed in Moonen Chrit and its prior art (mainly publications and patents by the present inventor).

The normal events occurring in cells before, during and after stress are described on p.2, lines 5-12: "HSF1 is continuously and ubiquitously expressed in mammalian cells. In the absence of stress, the factor is present in an inactive form, unable to bind HSE sequences of stress gene promoters and to enhance their transcription. During stress, HSF1 is activated, and in the activated form, it binds HSE DNA and stimulates transcription of stress genes. Subsequent to a stressful event, the factor relatively rapidly returns to its inactive form. Consequently, transcription of stress genes ceases. Mutant forms of HSF1

have been constructed that are capable of constitutively transactivating stress genes, in the absence of stress."

See p.2, lines 22-25: "A major drawback of the use of stress promoters to control regulation of a gene of interest is that gene expression by a stress promoter can be maintained beyond the duration of the stress treatment only under conditions of extreme stress. Yet such extreme conditions are incompatible with cell survival."

See p.12, lines 2-5: "Several regulatory circuits are described herein that have the general feature that their use permits sustained activation of expression of a gene of interest by a single application of stress. Gene activity regulated by these circuits does not subside subsequent to the activating stress."

It should be obvious from these passages that the invention could not possibly contemplate using endogenous HSF as a transcription factor component of its circuits. The very purpose of the invention is to correct the shortcomings of heat shock promoter-directed expression of genes of interest under the control of endogenous HSF.

delivered into a cell

Proposed amended claim 1, solution B

2. A molecular circuit **for delivery into a cell (or to be delivered into a cell)** comprising (a) a first nucleic acid molecule that comprises a gene encoding a transcription factor and a first promoter activatable by stress and by the transcription factor, wherein the first promoter and the transcription factor gene are operably linked, and (b) a second nucleic acid molecule that comprises a gene of interest and a second promoter activatable by the transcription factor, wherein the second promoter and the gene of interest are operably linked.

For literal support of the qualification, see p.12, lines 10-11. See also p.13, lines 19-25; p.23, lines 17-20, p25, lines 23-26 and p26, lines 3-5, and elsewhere.

RV, 8/5/01